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The Pancreatic Epithelial Cell *in Vitro*: A Possible Model System for Studies in Carcinogenesis[1]

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Summary

A technique is described for the isolation and maintenance in culture of pancreatic epithelial cells from human embryos or neonatal guinea pigs. Dissociated cells from human material were essentially uncharacterized. Those obtained from guinea pig pancreas were subjected to centrifugal fractionation, and the resulting populations consisted of up to 95% exocrine cells. Suspensions of dissociated cells were incubated with gyration for 16 to 18 hr, and cell aggregates that formed were placed in stationary culture. Two-dimensional colonial aggregates developed on the plastic substratum. Cells comprising such colonies formed junctional complexes and could be maintained for 20 to 40 days *in vitro*. This system may provide a useful model for studies on pancreatic exocrine cell physiology and carcinogenesis.

Introduction

The development of techniques for maintaining viable pancreatic epithelial cells in culture would provide a substantial advantage for studies on a variety of problems related to pancreatic cell physiology. The progressive increase in incidence of pancreatic carcinoma (5) is one major concern in this regard, and a biological model system to screen for etiological agents effecting this would provide a valuable diagnostic tool. Since the highest incidence of pancreatic carcinoma involves neoplasms of ductal or acinar cell origin (6, 8), possible methods for isolation and maintenance of these cell types are being studied. Techniques for cultivation of endocrine pancreatic cells have been in use for some time (3, 7). This report summarizes recent efforts in this laboratory involving culture of acinar cells from guinea pig pancreatic tissue and uncharacterized epithelial cells from human embryonic pancreas.

Tissue Dissociation

Pancreatic tissue was obtained from aborted human fetuses, usually of 10 to 20 weeks gestation, or from postnatal guinea pigs weighing 250 g or less. Each organ was rinsed in Hanks' saline and thoroughly minced with lens scissors. The fragments were dissociated in a mixture of crude trypsin (0.125%) and collagenase (0.5%) by rapid agitation (120 rpm) for 15 min at 37°. The collagenase stock solution was prepared from crude material (Sigma type I) as described by Cahn *et al.* (2). Preliminary studies indicated that this procedure gave optimum cell yields per unit time when compared to several other dissociation mixtures. It also compared favorably when percentage of viability (by erythrosin B exclusion) and overall plating efficiencies were determined. The growth medium used throughout was Coon's modification of Ham's Medium F12 (2) supplemented with 15% selected fetal calf serum (for clonal culture or aggregation) or 20% selected calf serum (for colonial aggregate isolation). Standard techniques were utilized for clonal culture and for physical isolation of clones and colonial aggregates (2).

Although epithelial clones did develop from both guinea pig and human embryonic tissue, plating efficiencies were low and such clones did not achieve sufficient size or division potential for cell strain establishment. Epithelial cells in high yield could be recovered from primary suspensions, however, by introducing the aggregation procedure outlined below. Pancreatic epithelial cell aggregates from human tissue were obtained without the fractionation step (4).

Fractionation and Aggregation Procedure

A fractionation procedure for mixed primary cell suspensions was adapted from that of Amsterdam and Jamieson (1). Dissociated cells from guinea pig tissue were collected in Medium F12 with 15% fetal calf serum and filtered through nylon mesh of 20-μm pore size. The resultant cell suspension was dispersed further by mixing through a syringe fitted with an 18-gauge needle. The suspension was then layered over a solution containing 4% bovine serum albumin in Hanks' saline. In most cases the latter was set up in 30-ml amounts in 40-ml centrifuge tubes, and 3 to 4 ml of cell suspension (10^7 viable cells/ml) were added at the top. The tubes were then spun at $100 \times g$ for 5 min. This manipulation was performed 2 more times in succession and the resulting cell pellet, consisting of up to 95% acinar cells, was resuspended in Medium F12 containing 15% fetal calf serum and soybean trypsin inhibitor (0.1 mg/ml). The concentration of cells was adjusted to 2×10^6/ml, and the suspension was dispensed to 25-ml conical flasks in 5-ml aliquots for subsequent aggregation. The flasks were then placed in a gyrating water bath set at 37°, and the contents

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were permitted to aggregate for 16 to 18 hr at 80 rpm. The aggregates so formed were transferred to Medium F12 supplemented with 20% calf serum for seeding at 10^3 to 10^4 aggregates/cm of culture surface area.

Colonial Aggregate Formation and Maintenance

Fig. 1*A* depicts a typical aggregate formed after 18 hr of gyration. Similar conglomerates were obtained with either guinea pig or human source material, but zymogen-like droplets were not prominent in cells from the latter (4). Such 3-dimensional aggregates attach to the substrate when placed in stationary culture and spread out in radial fashion to form colonial aggregates of epithelial cells (Fig. 1*B*). Amylase has been detected within such cell groupings prepared from guinea pig material for periods up to 5 days after dissociation (4). Activity declines rapidly, however, during this maintenance period. Progressive decrease in tissue-specific enzyme synthesis is probably not related to cell surface damage since aggregates have been shown to be responsive to pancreozymin even at 24 hr after dissociation (4).

Epithelial cell survival may be enhanced by reformation of tight junctions, which have been noted at 72 hr after plating (Fig. 1*C*). Typical maintenance periods of 20 to 40 days were possible for colonial aggregate cultures from both species. At terminal stages the cells became more rounded and refractile, and they eventually detached from the substrate (4). This limited maintenance period may be sufficient to permit screening trials for carcinogenic potential. Nevertheless, our present working hypothesis predicates that common existent culture conditions, physical, humoral, or nutritional, are inadequate to support continued active proliferation and function of this cell type. Current research efforts are directed toward identification of these hypothetical deficiencies.

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References

1. Amsterdam, A., and Jamieson, J. D. Structural and Functional Characterization of Isolated Pancreatic Exocrine Cells. Proc. Natl. Acad. Sci. U. S. A., *69:* 3028-3032, 1972.
2. Cahn, R. D., Coon, H. G., and Cahn, M. B. Cell Culture and Cloning Technique. *In:* F. H. Wilt and N. K. Wessels (eds.), Methods in Developmental Biology, pp. 493-530. New York: Crowell Co., 1966.
3. Chick, W. L., Lauris, V., Flewelling, J. H., Andrews, K. A., and Woodruff, J. M. Effects of Glucose on Beta Cells in Pancreatic Monolayer Cultures. Endocrinology, *92:* 212-218, 1973.
4. Hay, R. J. Proliferative and Functional Impairment of Pancreatic Epithelial Cells Maintained *in Vitro*. *In:* V. J. Cristofalo and E. Holeckova (eds.), Cell Impairment in Aging and Development, pp. 23-39. New York: Plenum Press, 1975.
5. Levin, D. L., and Connelly, R. R. Cancer of the Pancreas. Available Epidemiological Information and its Implications. Cancer, *31:* 1231-1236, 1973.
6. Miller, J. R., Baggenstoss, A. H., and Comfort, M. W. Carcinoma of the Pancreas. Effect of Histological Type and Grade of Malignancy on Its Behavior. Cancer, *4:* 233-241, 1951.
7. Orci, L., Like, A., Amherdt, M., Blondel, B., Kanazawa, Y., Marliss, E. B., Lambert, A. E., Wollheim, C. B., and Renold, A. E. Monolayer Cell Culture of Neonatal Rat Pancreas: Ultrastructure and Biochemical Study of Functioning Endocrine Cells. J. Ultrastruct. Res. *43:* 270-297, 1973.
8. Silen, W. Pancreas. *In:* S. I. Schwartz (ed.), Principles of Surgery, pp. 1255-1280. New York: McGraw-Hill Book Co., 1969.

Fig. 1. *A*, aggregate of acinar cells from guinea pig pancreas 18 hr after tissue dissociation. Note prominent zymogen droplets (*arrow*). Phase contrast, × 760. *B*, colonial aggregate, formed from aggregate as shown in *A*, 96 hr after dissociation. Phase contrast, × 210. *C*, electron micrograph showing tight junctions between cells of a colonial aggregate 96 hr after dissociation. Sections were cut tangential to culture surface. × 15,168.